

OXALIC ACID IN FOODS AND ITS BEHAVIOR AND FATE IN THE DIET

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THREE FIGURES

(Received for publication April 25, 1939)

One is apt to gather from nutritional literature that vegetables do not supply calcium efficiently. Because this is not in accord with the results of a number of experiments, some covering several generations of animals (Kohman, Eddy and associates, '31, '34), this discrepancy presented itself as a problem wanting solution. As there seemed to be confusion about the amount of oxalates in foods and a scarcity of data as to the behavior and fate of oxalates in the diet, information on these points might be expected to throw light on the availability of calcium. It seemed desirable for higher accuracy, to make oxalate determinations under conditions that would avoid drastic treatment of the food and entail the concentration of the oxalate of a relatively large sample, freed from most of the other food constituents.

METHOD OF ANALYSIS

The following procedure was used. The samples, 400 gm., were first cooked tender by steaming without drainage loss. After thorough disintegration and an approximation of water content, sufficient concentrated hydrochloric acid was added to yield a 15% solution in the water present. This was

¹ The experimental work embodied in this paper was done in 1934-1936 while the author was with the Research Laboratory of the National Canner's Association, Washington, D. C. There he had the assistance of N. H. Sanborn and D. C. Smith. This opportunity is taken to acknowledge their part in the work.

allowed to stand at room temperature, with occasional stirring, not less than 2 days.

The sample was then pressed in cheese cloth and the weights of the drained material and of the residue recorded. By direct titration of a highly diluted sample, after filtration if necessary, the chlorine in the two fractions was determined. On the assumption that the oxalic acid distributed itself in the same ratio as the chlorine, it could be calculated for the entire sample by a determination on the drained fraction. This assumption was proved correct for pineapples and turnip greens by adding varying amounts of oxalic acid and subsequent analysis.

A 250 to 300 cc. sample of the drained liquid was subjected to 24 hours of wet ether extraction in an apparatus modeled after that suggested by Palkin, Murray and Watkins ('25) and suitably proportioned for this purpose using sintered glass to disperse the ether. In this the liquid to be extracted was 32 to 37 cm. deep and the ether coursed up through it from the 2.6 sq.cm. of fritted glass at a rate of 20 cc. per minute. When the extraction was complete, water was added to the extract, the ether evaporated and the water solution filtered. Calcium oxalate was then precipitated. After washing and redissolving a second, and if necessary, a third precipitation was made for complete purification. The precipitate was then titrated with permanganate. By precipitating the calcium in the titrated medium as calcium oxalate, the purity of the original oxalate could be gauged. All determinations were made in duplicate and the average is given in table 1. To study varietal and other factors more than one sample of some products were analyzed. As no significance could be placed on the differences, all the results were averaged and the number of samples is given in parenthesis.

THE OCCURRENCE OF OXALATES IN FOODS

Only a few foods, notably spinach, Swiss chard, New Zealand spinach, beet tops, lamb's quarter, poke, purslane and rhubarb have a high oxalate content. In them, expressed

TABLE 1
Oxalic acid (anhydrous) in foods

<i>Product</i>	<i>Total solids</i>	<i>Oxalic acid</i>	<i>Calcium</i>
	<i>%</i>	<i>%</i>	<i>%</i>
Vegetables			
Asparagus	6.05	0.0052	0.0201
Beans, green pod (3)	9.58	0.0310	0.0440
Beans, wax	7.80	0.041	0.054
Beans, lima (2)	24.25	0.0043	0.044
Beets, unpeeled	8.23	0.138	0.018
Beet leaves	6.60	0.916	0.120
Beet stems	6.66	0.338	0.040
Broccoli, leaves and flowers	10.50	0.0054	0.21
Broccoli, stalks	7.57	0.0035	0.092
Cabbage (2)	8.80	0.0077	0.189
Cabbage sprouts	8.52	0.0059	0.150
Cabbage, Chinese	6.45	0.0073	0.210
Carrots	11.02	0.033	0.044
Cauliflower	8.90	None	0.034
Celery stalks, bleached	4.58	0.034	0.054
Celery, soup leaves	14.66	0.050	0.55
Celery, soup stems	10.20	0.062	0.18
Collards (2)	12.75	0.0091	0.361
Chard, Swiss, leaves	9.47	0.66	0.11
Chard, Swiss, stalks	7.10	0.29	0.045
Chard, Swiss, leaves and stalks (2)	8.28	0.645	0.129
Chenopodium (Lamb's quarter)	8.20	1.11	0.099
Corn, sweet, white	25.00	0.0014	0.0076
Corn, sweet, yellow (2)	33.51	0.0052	0.0033
Cress, land, wild	15.00	None	0.24
Cress, early fine curled	8.80	0.0106	0.182
Cucumbers	3.72	None	0.014
Dandelions (3)	11.38	0.0246	0.171
Egg plant	6.18	0.0069	0.010
Endive (5)	7.58	0.0273	0.105
Escarole	6.10	0.0116	0.087
Kale	11.05	0.013	0.31
Kale, minus leaf ribs	18.05	0.011	0.294
Lettuce (6)	6.46	0.0071	0.073
Mustard greens (3)	8.40	0.0077	0.235
Okra	13.20	0.048	0.077
Onions, green	13.65	0.023	0.057
Parsley	13.70	0.19	0.29
Parsnips	22.70	0.010	0.049
Peas	19.50	None	0.019
Peppers, sweet, green (3)	7.34	0.016	0.0135
Poke	7.74	0.476	0.052
Potatoes, Irish	20.38	0.0057	0.0094
Potatoes, sweet	33.60	0.056	0.034
Purslane, leaves	9.45	0.910	0.13
Purslane, stalks	8.44	0.518	0.067
Radishes	3.75	None	0.028

TABLE 1—Continued

<i>Product</i>	<i>Total solids</i> %	<i>Oxalic acid</i> %	<i>Calcium</i> %
Vegetables			
Rape	10.82	0.0015	0.11
Rhubarb	6.62	0.50	0.044
Spinach (53)	10.35	0.892	0.122
Spinach, canned (12)	7.15	0.364	0.058
Spinach, New Zealand, leaves	7.60	0.89	0.11
Spinach, New Zealand, stalks	8.26	0.65	0.083
Squash, green summer	5.51	None	0.036
Turnips, peeled	8.16	None	0.037
Turnips, unpeeled	6.58	0.0018	0.028
Turnip greens	8.25	0.0146	0.245
Fruits			
Apples, early summer	12.58	None	0.010
Apricots	13.62	0.014	0.024
Avacados	14.60	None	0.0095
Bananas	23.81	0.0064	0.0071
Berries, black	12.25	0.018	0.038
Berries, blue	20.81	0.015	0.026
Berries, black rasp	22.10	0.053	0.058
Berries, dew	13.70	0.014	0.027
Berries, green goose	13.05	0.088	0.023
Berries, red rasp	14.10	0.015	0.023
Berries, straw	10.48	0.019	0.031
Cherries, red sour	12.18	0.0011	0.010
Cherries, sweet, Bing	24.50	None	0.0019
Currants, red	15.52	0.019	0.030
Grapes, Concord	15.30	0.025	0.024
Grapes, Thompson's seedless	23.90	None	0.013
Grapefruit	11.50	None	0.015
Lemons, juice	9.20	None	0.011
Lemons, peel	18.90	0.083	0.17
Limes, juice	10.39	None	0.015
Limes, peel	31.00	0.11	0.26
Mangoes	15.35	None	0.015
Melons, cantaloup	8.46	None	0.0090
Melons, casaba	11.22	None	0.0054
Melons, honey dew	6.08	None	0.0090
Melons, water	10.42	None	0.0060
Nectarines	14.45	None	0.0084
Oranges, edible portion	15.15	0.024	0.038
Oranges, peel	22.90	0.078	0.15
Peaches, Alberta	15.68	0.0050	0.012
Peaches, Hiley	14.10	None	0.0089
Pears, Bartlett	17.60	0.0030	0.014
Pineapples, Hawaiian canned	17.44	0.0063	0.019
Plums, damson	11.70	0.010	0.015
Plums, green gage	13.20	None	0.0080
Prunes, Italian	15.76	0.0058	0.12
Tomatoes	5.76	0.0075	0.010

as anhydrous oxalic acid, it is often considerably over 10% on a dry basis. One per cent in table 1 is equivalent to 1.4% of the usual crystalline form of oxalic acid. In most foods there are present mere traces. It is notable, however, that oxalate was obtained from all but a very few products, mostly fruits.

One purpose of the analytical survey was to ascertain if some varieties of spinach might be relatively free of oxalate. This hope was not realized. In fifty-three samples, including practically all commercial and many experimental varieties grown in California and in Maryland as well as those shipped from Texas, Florida and Carolina, the average anhydrous oxalic acid content was 9.02% on the dry basis (maximum 12.6, minimum 4.5). The calcium values averaged 1.25% (maximum 2.50, minimum 0.44). California spinach was only slightly lower in oxalic acid but markedly lower in calcium. California spinach averaged 0.59% calcium (maximum 0.84, minimum 0.44) while Maryland spinach averaged 1.92% (maximum 2.50, minimum 1.42). Since a considerable number of mineral elements are dietary essentials, such a variation raises a number of important questions.

PLAN OF FEEDING EXPERIMENTS

As a number of widely used greens contain insignificant amounts of oxalates, a comparison of these with spinach seemed in order. Green leaf-vegetables are our richest calcium sources. In previous experiments Kohman, Eddy and White ('37) used diets of canned foods and found them to supply calcium efficiently. For convenience, therefore, a basal diet of one can each of roast beef, peas, carrots and sweet potatoes was chosen. The essential analysis expressed in grams was:

<i>One can</i>	<i>Net weight</i>	<i>Solids</i>	<i>Calcium</i>	<i>Phosphorus</i>
Roast beef	362	133	0.054	0.155
Peas	607	111	0.106	0.474
Carrots	599	45	0.147	0.156
Sweet potatoes	551	184	0.133	0.251

By securing a sufficient amount from one lot of each of these, it was possible to have a constant, uniform supply for

an entire series of experiments conducted over an extended period of time. These four foods, in the above proportion, were thoroughly mashed and mixed, liquid and all, and hence had to be eaten in that proportion. This diet was obviously low in calcium, i.e., 0.093%. It permitted good but not maximum growth and bone formation, evincing efficient calcium. Small additions of greens (5 to 8%) were made to supply 60% of the calcium of the final mixture. This raised the calcium content to 0.22%.

In experiment 3, table 2, some additions to the basal diet were on a different basis. The turnip greens in diet C supplied only 58.8% of the calcium. It was added in that amount to be compared with diet D, in which it was accompanied by an equal amount of spinach, which was half the amount of spinach added in diet B to supply 60% of the calcium. The two greens in diet D supplied 70% of the calcium and raised the content to 0.3%. Also in experiment 3, diet D was planned to be equal to diets B and E on the basis of crude fiber, since the effect of crude fiber on calcium availability has often been questioned. Diet E is equal to D both in fiber and calcium.

The rats used were purchased from a large dealer who was able to supply large litters suitable for apportioning between the diets of a given experiment equally as to weight and sex. They were started on the experiments at 21 or 22 days of age when they weighed 30 to 35 gm. Six animals were always placed on each diet and kept in one cage with a raised wire bottom, three mesh to the inch. Except as noted otherwise the duration of the experiments was 21 days. There was an occasional death during this period and in every case it was an animal receiving either spinach or calcium oxalate.

RESULTS OF FEEDING EXPERIMENTS

It is apparent from data in tables 2, 3 and 4 that whereas spinach greatly increases the calcium content of the low calcium but well performing basal diet, it decidedly interferes with both growth and bone formation. This cannot be ex-

TABLE 2

Growth of rats and bone ash of alcohol-ether extracted dry tibia

<i>Dietary addition</i>	<i>Weight gain</i>		<i>Ash in tibia</i>
Experiment 1—21 days			
	<i>gm.</i>		<i>%</i>
Turnip greens (4.5%)	59		43.2
Spinach (8.2%)	38		33.4
Experiment 2—28 days			
None	67		44.7
Calcium carbonate	94		50.0
Calcium oxalate	79		47.6
Turnip greens (4.5%)	106		51.6
Spinach (8.2%)	77		45.2
Experiment 3—21 days			
None	Diet A	54	44.1
Spinach (8.8%—23.24 gm.)	Diet B	40	37.8
Turnip greens (4.4%—11.62 gm.)	Diet C	87	53.1
Turnip greens 11.62 gm. + spinach 11.62 gm.	Diet D	74	47.9
Spinach 23.24 gm. + CaCO ₃ 0.406 gm.	Diet E	46	39.5
Experiment 4—21 days			
None		53	45.3
Canned spinach brand 1		47	40.3
Canned spinach brand 2		58	40.9
Canned spinach brand 3		57	43.5
Freshly cooked spinach		62	41.1
Experiment 6—21 days			
Calcium carbonate		76	51.1
Spinach, + CaCO ₃ equivalent to oxalate in spinach		88	53.4
Spinach (7%)		57	40.5
Kale (6%)		79	53.5
Mustard greens (5%)		84	53.2
Experiment 7—21 days			
None		52	47.3
Canned spinach brand 1—not drained		45	45.7
Canned spinach brand 1—drained		54	45.1
Canned spinach brand 3—not drained		52	44.8
Canned spinach brand 3—drained		56	46.0
Freshly cooked spinach—not drained (9%)		54	45.5
Freshly cooked spinach—drained (7%)		75	49.1

plained on the basis of crude fiber. On the other hand greens with negligible oxalate content, such as turnip greens, kale, mustard greens and collards, markedly improve growth and bone formation under similar conditions. If enough calcium carbonate is added with the spinach to balance stoichiometrically its oxalate, performance in the rat is then comparable with other greens. This would require, in the fifty-three samples analyzed, from two to nine times as much calcium as the spinach contains. A general impression was obtained that some superiority, such as sleekness of fur, resulted when a low oxalate bearing green supplied calcium as against an equivalent addition of calcium carbonate.

In table 2 are recorded the results of a series of experiments in which the gain in weight and the per cent ash in the tibia was determined after feeding the basal diet alone and also the basal diet with various additions, to similar groups of animals. Each figure represents the average of six animals.

Table 3 records the gain in weight and the gain in calcium per animal in other similar feeding experiments. To arrive at the calcium per animal at the time the feeding was started, a similar group of six animals was sacrificed and their calcium content determined. In all cases when an entire animal was analyzed, the food was removed the evening before, i.e. 16 hours. In experiment 8, table 3, the food consumption was determined and from this the calcium utilization obtained. It appears that when turnip greens were added to the basal diet 79% of the calcium was utilized but when spinach was added only 15% was utilized. Assuming that the calcium supplied by the basal diet and the turnip greens was equally available, and bearing in mind that 15% of the total calcium is only 38% of that supplied by the basal diet, it appears that the spinach not only supplied no available calcium, but it actually rendered unavailable 41% of the calcium of the basal diet, i.e., the difference between 79 and 38. The sample of spinach used had 10.1% oxalic acid and 1.53% calcium. The diet to which it was added then had 0.8% oxalic acid. When calcium oxalate was the addition, 44% of the calcium was

utilized, showing that the rat can make use of a small portion of the calcium in it. It is notable that with turnip greens in the diet four times as much calcium per gram of tissue was deposited as with spinach in the diet. The data in tables 3 and 4, experiment 8, indicate a superiority of turnip greens over calcium carbonate in growth, calcium utilization and calcium deposition per unit body weight.

TABLE 3
Growth record and gain in calcium

<i>Addition to basal diet</i>	<i>Gain in weight</i>	<i>Calcium per rat</i>		<i>Calcium gain per gram gain in weight</i>	<i>Calcium utilized</i>
		<i>Total</i>	<i>Net gain</i>		
Experiment 5—21 days					
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>%</i>
Controls—21 days old	—	0.1853	—	—	—
Calcium oxalate	62	0.3535	0.1682	0.00267	—
Spinach (8.2%)	56	0.2449	0.0596	0.00106	—
Turnip greens (4.5%)	84	0.5300	0.3447	0.00410	—
Experiment 8—21 days					
Controls—21 days old	—	0.2172	—	—	—
Calcium carbonate	71	0.4205	0.2033	0.00286	70
Calcium oxalate	56	0.3286	0.1115	0.00199	44
Spinach	37	0.2447	0.0275	0.00074	15
Turnip greens	81	0.4603	0.2431	0.00300	79

RECOVERY OF CALCIUM AND OXALIC ACID IN URINE AND FECES

In a number of these experiments the calcium and oxalate excreted in the urine and feces were determined in addition to the calcium and oxalate in the food consumed and the calcium deposited in the tissue. This included any endogenous oxalic acid which would be overshadowed by the spinach oxalate. The data are recorded in table 4. The collection period was 7 or 8 days duration, thus allowing two periods in a 21-day experiment. The collection was begun 3 or 4 days after the animals were placed on a diet. There seems to be a tendency for less oxalic acid recovery in the second period, indicating that the ability to oxidize it increases with age. Also there is better utilization of calcium in the second period.

While urinary calcium may not seem high with spinach feeding, it represents a relatively high percentage of assimilated calcium, probably passing as oxalate through the excretory organs.

In only one case with spinach in the diet was as much of the calcium utilized as was supplied by the basal diet. The ex-

TABLE 4
Recovery of oxalic acid and calcium

<i>Dietary addition</i>	<i>Per cent oxalic acid recovered</i>			<i>Per cent calcium recovered</i>			<i>Calcium utilized</i>
	<i>In urine</i>	<i>In feces</i>	<i>Total</i>	<i>In urine</i>	<i>In feces</i>	<i>Total</i>	
Experiment 2							
Calcium oxalate	8	39	47				%
Spinach	10	23	33				
Experiment 3							
Spinach {	Period 1	14	77	91			
	Period 2	18	72	90			
Spinach + CaCO ₃ {	Period 1	12	84	96			
	Period 2	12	78	90			
Experiment 5							
Spinach {	Period 1	14	55	69			
	Period 2	10	34	44	10	60	70
Calcium oxalate {	Period 1	6	47	53			
	Period 2	6	34	41	3	33	36
Experiment 7							
Spinach not drained {	Period 1	13	50	63	2	86	88
	Period 2	11	23	34	4	62	66
Spinach drained {	Period 1	8	57	65	2	57	59
	Period 2	6	27	33	3	40	43
Experiment 8							
Calcium carbonate {	Period 1				18	8	26
	Period 2				9	6	15
Calcium oxalate {	Period 1	13	55	68	3	43	46
	Period 2	6	73	79	3	63	66
Spinach {	Period 1	11	33	44	3	73	76
	Period 2	8	24	32	8	62	70
Turnip greens {	Period 1				10	8	18
	Period 2				3	6	9

ception is in table 4, experiment 7. In this the spinach was cooked by dropping it in twice its weight of boiling water, allowing 7 minutes to return to boiling and then boiling 10 minutes. After draining, its weight was 58% of the original. The undrained spinach was cooked 20 minutes in its adhering water. There was, however, very poor calcium utilization even with drained spinach. Numerous experimental and commercial blanching tests showed it is difficult to remove more than half the oxalate by this process. Spinach calcium is insoluble and hence not extractable.

EXTENDED FEEDING PERIOD

In one experiment twelve animals at 21 days of age were placed on each of two diets. In one group spinach supplied 60% of the calcium and in the other turnip greens. By the time the age of 90 days was reached, five animals on the spinach diet had died while all those on the diet containing turnip greens were in excellent condition. The average weight at 90 days of age of animals receiving spinach was 134 gm. while of those receiving turnip greens it was 205.

In due time two litters of nine young each appeared on the diet with turnip greens. These were reared to the age of 21 days when the average weight was 39 gm. in one litter and 34 in the other, while the average weight of their parents at the same age was 26 gm. Some time later one litter appeared among the animals on the diet with spinach, all but two of which were dead and these were shortly eaten by their mother.

Figures 1, 2 and 3 are photomicrographs of a tooth of an animal respectively on each of the three diets in experiment 3, table 2, i.e., the basal diet alone, with spinach added and with turnip greens. The band across which the line is drawn represents the dentine layer of a rat's tooth magnified 310 times. The dark portion of this band designated 'A' is calcified while the light portion 'B' is uncalcified and represents the area where new dentine is being formed. The wide uncalcified area in figure 2, representing the spinach diet is

apparent. It should be mentioned here that the bones of animals receiving spinach were very soft and pliable.

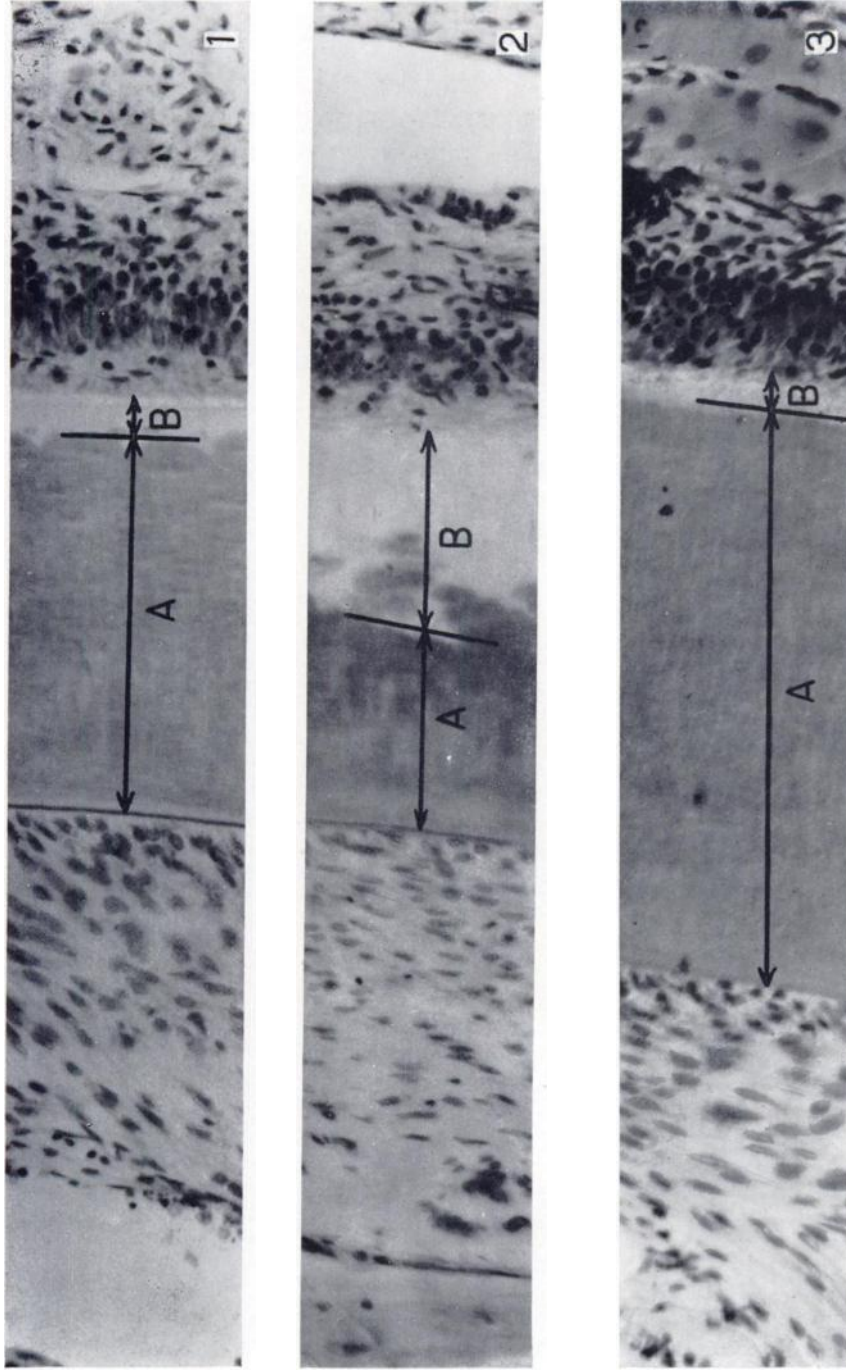
DISCUSSION

Insofar as these data are on a comparable basis they are in agreement with the reports of Tisdall and Drake ('38), Fairbanks and Mitchell ('38) and Fincke and Sherman ('35), published since this work was done. They all dealt with calcium availability. No one seems to have studied heretofore the fate of the oxalates in the diet of the rat. This is perhaps of equal importance. Some oxalic acid is assimilated and tends to carry calcium with it into the urine. In view of the low solubility of calcium oxalate, there arises the question of any possible damage of its passage through the excretory tissues. The demonstration of almost universal presence of small amounts of oxalates in vegetables and fruits tends to throw doubt on the quantitative aspects of endogenous oxalic acid.

CONCLUSION

Oxalates, expressed as anhydrous oxalic acid, have been shown to occur to the extent of about 10% on a dry basis in spinach, New Zealand spinach, Swiss chard, beet tops, lamb's quarter, poke, purslane and rhubarb. Traces were found in nearly all vegetables and fruits.

If to a diet of meat, peas, carrots and sweet potatoes, relatively low in calcium but permitting good though not maximum growth and bone formation, spinach is added to the extent of about 8% to supply 60% of the calcium, a high percentage of deaths occurs among rats fed between the age of 21 and 90 days. Reproduction is impossible. The bones are extremely low in calcium, tooth structure is disorganized and dentine poorly calcified. Spinach not only supplies no available calcium but renders unavailable considerable of that of the other foods. Considerable of the oxalate appears in the urine, much more in the feces.



Photomicrographs, $\times 310$. Dentine of rat's tooth; 'A' calcified, 'B' uncalcified area. Figures 1, 2, and 3 respectively from animal receiving basal diet only, basal diet with spinach and basal diet with turnip greens, diets A, B and C respectively in experiment 3, table 2.

Turnip greens, mustard greens, kale and collards, greens with negligible oxalates, under similar conditions produce excellent animals that deposit four times as much calcium per unit body weight as those receiving spinach.

Acknowledgment is made to Capt. A. L. Irons, Dental Corps, U. S. A., who prepared the photomicrographs, and to the experiment stations at Davis, California, Beltsville, Maryland and Geneva, New York, where various samples were obtained.

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